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Title Of FACT complex and oxidative stress response: a KEAP1/NRF2-dependent novel mechanism sustaining hepatocellular carcinoma progression

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Abbreviations

ARE: Antioxidant Responsive Element; BACH1: BTB Domain and CNC Homolog 1; FACT: Facilitates Chromatin Transcription; HCC: Hepatocellular carcinoma; KEAP1: Kelch Like ECH Associated Protein 1; NAFLD: non-alcoholic fatty liver disease; NQO1: NADPH Quinone Dehydrogenase 1; NRF2: Nuclear factor erythroid 2 – Related Factor 2; ROS: Reactive Oxygen Species; SSRP1: Structure Specific Recognition Protein 1; SUPT16H: Suppressor of Ty 16 Homolog; TKT: Transketolase; TXNRD1: Thioredoxin Reductase 1.

Keywords

Hepatocellular carcinoma, FACT complex, NRF2, KEAP1, Reactive oxygen species

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Hepatocellular carcinoma (HCC) is the most common primary liver cancer that usually develops in cirrhotic patients, except for progressive nonalcoholic fatty liver disease (NAFLD, where the tumor may develop also in non-cirrhotic liver) [1]. HCC is currently the leading cause of mortality in cirrhotic patients, representing the fifth most common cancer and the second leading cause of cancer mortality worldwide [1]. Although screening programmes can allow to identify HCC at an earlier stage in patients at risks, still a minority of patients can survive at 5 years from diagnosis, despite treatment. Current treatment options have limitations and first line drugs approved for systemic therapy, like sorafenib and lenvatinib, can at best offer additional 3 months of survival to HCC patients, emphasizing the urgent need to identify novel molecular targets to develop more effective therapies [1].

Chronic liver disease progression towards HCC development as well as HCC progression are highly affected by microenvironmental cues in a very complex scenario involving inter-relationships between cells (cancer cells, tumor associated macrophages or fibroblasts and cancer stem cells) as well as processes or events like inflammatory response, fibrogenic progression, autophagy, hypoxic conditions and oxidative stress [2]. In particular, an increase in intracellular levels of reactive oxygen species (ROS) represents a common feature of cancer cells which is usually counterbalanced by an up-regulation of antioxidant defenses, particularly through the relevant KEAP1 (Kelch Like ECH Associated Protein 1) and NRF2 (Nuclear factor erythroid 2 – Related Factor 2) - pathway [3]. In this pathway KEAP1, a E₂-ligase which is normally negatively regulating NRF2 protein stability via ubiquitin–proteasome degradation, is inactivated under oxidative stress; this preserves NRF2 from degradation and allows its nuclear translocation and binding to antioxidant response element (ARE) sequences in the promoter of antioxidant genes, displacing BACH1 (BTB Domain and CNC Homolog 1, the selective competing transcription repressor). This is a critical issue since although very high levels of intracellular ROS may even induce cancer cell death (apoptotic or necroptotic), lower levels of intracellular ROS sustained throughout the time can be pro-carcinogenic by inducing oxidative damage to DNA (favoring

development of new mutations and cancer progression) or by altering signal transduction and transcriptional control, two additional critical mechanisms underlying cancer cell response to microenvironmental cues.

In this issue of Gut an elegant experimental and clinical study by Shen et al. [4] elucidates the close and targetable relationships existing between the occurrence of oxidative stress, KEAP1/NRF2 - dependent antioxidant response and the activity of the Facilitates Chromatin Transcription (FACT) complex, a histone chaperone. As it is well known, histone chaperones play a fundamental role in controlling, together with ATP-dependent chromatin remodelers and histone-modifying enzymes, dynamic changes of chromatin like active disassembly, reassembly and reposition of nucleosomes, being then critical for DNA replication, DNA damage repair and gene transcription [5,6]. Not surprisingly, a deregulation of the interrelated activities of factors involved in the control of dynamic chromatin changes can affect genome stability and gene expression, overall promoting the development of several diseases, including cancer [5,6]. Human histone chaperone FACT complex is an heterodimer composed by the two subunits SUPT16H (Suppressor of Ty 16 Homolog, 140 kDa) and SSRP1 (Structure Specific Recognition Protein 1, 80 kDa) that is involved in almost all chromatin-related processes [7]. Although FACT complex has been recently reported to be deregulated in breast cancer and potentially targetable [8], the mechanism(s) by which its deregulation may contribute to cancer progression is(are) still unknown and data on human HCC are lacking.

In their study Shen et al. [4] first analyzed HBV-associated human HCC specimens and their corresponding non-tumorous (NT) livers and then different human HCC cell lines and TCGA human HCC cohort of mixed etiology, obtaining the following major findings: a) deregulation of histone chaperones was a very common event in human HCC; b) both subunits of the FACT complex, SUPT16H and SSRP1, were significantly up-regulated in human HCC specimens; c) SUPT16H and SSRP1 levels were also associated with poor overall survival and disease-free survival of HCC patients. The relevance of these human findings let Authors to investigate the

pro-carcinogenic role of FACT complex. By employing a novel CRISPR/Cas9 SAM (Synergistic Activation Mediator) system Authors found that simultaneous up-regulation of both SUPT16H and SSRP1 subunits significantly promoted cell proliferation, colony formation and cell migration in human MHCC97L cells. In vivo overexpression of FACT complex significantly promoted HCC tumor growth in the subcutaneous implantation nude mice model, suggesting that indeed FACT complex may operate as an oncogenic complex. Knockout of SUPT16H or SSRP1 confirmed the hypothesis by significantly suppressing HCC cell proliferation, migration and colony formation in MHCC97L as well as HCC growth and lung metastasis in a murine orthotopic implantation model. Of interest, knockout of one subunit led to degradation of the other, suggesting that FACT complex stability in HCC strictly depend on subunits interaction.

The analysis of promoter sequences of SUPT16H and SSRP1 genes led Authors to focus on NRF2 and BACH1 [9] and, by employing mechanistic experiments, to reveal a novel critical link between FACT complex and KEAP1/NRF2 pathway. Authors provide data indicating that at protein level FACT complex expression is regulated by KEAP1-mediated protein degradation but at transcript level FACT complex is dependent on NRF2-mediated action resulting in rapid transcription of target antioxidant genes like NQO1 (NADPH Quinone Dehydrogenase 1), TXNRD1 (Thioredoxin Reductase 1) and TKT (Transketolase) in response to oxidative stress. This means that FACT complex is essential for cancer cells to adapt and survive to the increased intracellular generation of ROS, a notion that is intrinsically relevant since KEAP1/NRF2 pathway is one of the most frequently mutated in human HCC and is associated with drug resistance [10].

A final extremely relevant and potentially translatable finding is based on experiments showing that treatment with Curaxin, a drug that traps FACT complex into chromatin, or knockout of FACT complex were both effective strategies to sensitize cancer cells to sorafenib action in HCC cell lines and in vivo tumor growth in subcutaneous implantation. In particular, cotreatment of Curaxin and sorafenib led to increased apoptosis of cancer cells and inhibition of proliferation [4].

In conclusion (Figure 1), this excellent study provides compelling and mechanistic evidence, potentially translatable to human HCC, that cancer cells can adapt and survive to increased intracellular ROS levels in HCC progression as well as during sorafenib treatment (which has been shown to increase oxidative stress by itself) through a ROS-mediated up-regulation of FACT complex (associated to its increased stabilization) resulting in the acceleration of the transcription elongation of NRF2 and related target antioxidant genes. Of relevance, this study indicates FACT complex as a reliable and specific marker for HCC detection and prognosis that can be selectively and efficiently targeted by curaxin. Moreover, curaxin-mediated inhibition of FACT complex not only strongly compromised the oxidative stress adaptive response but also significantly sensitized HCC cancer cells towards the action of the first line drug sorafenib, opening the way to a novel and potentially more efficient strategy of therapeutic intervention for HCC patients.

Footnotes

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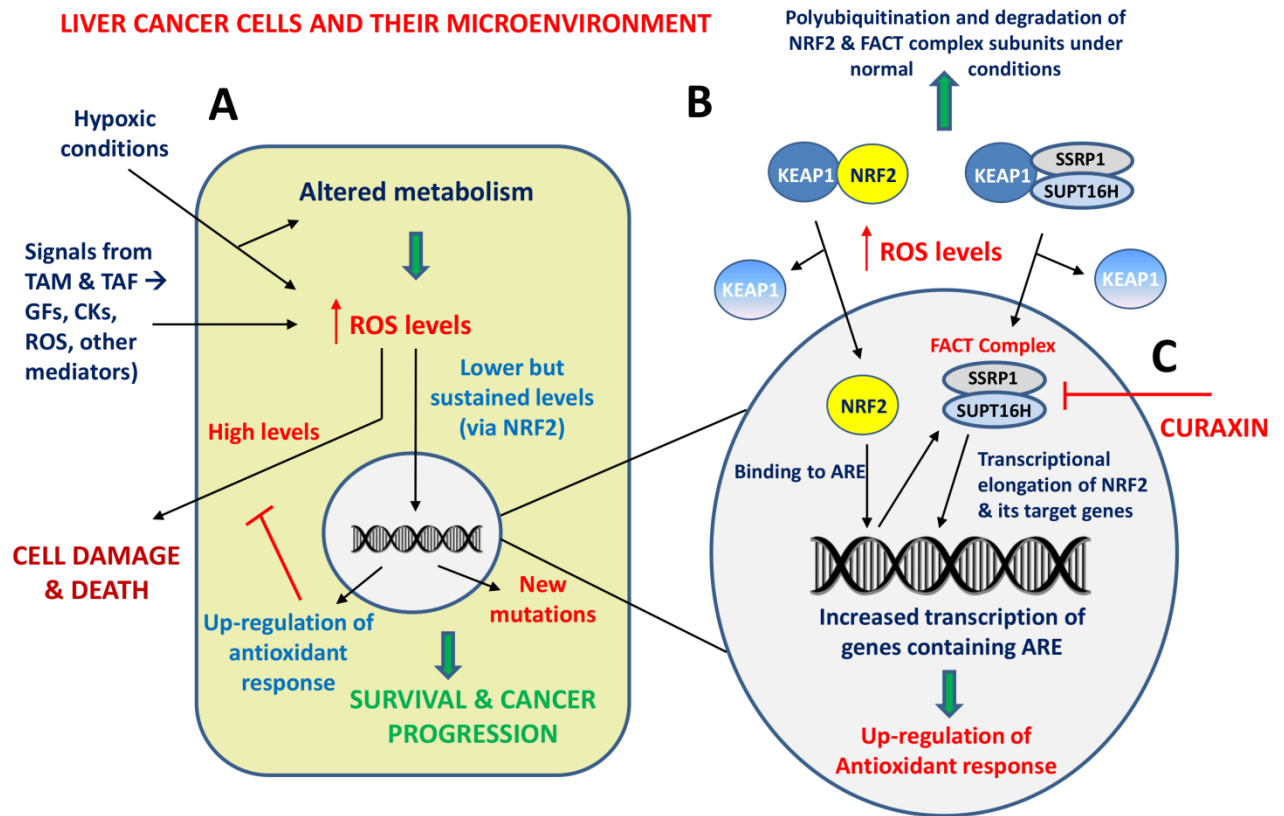


Figure 1. The relationships between NRF2/KEAP1 pathway, FACT complex and antioxidant response in liver cancer cells. (A) Liver cancer cells are commonly exposed to a significant increase of intracellular ROS levels, as a consequence of altered metabolism and/or of signals coming from the surrounding microenvironment, particularly from tumor-associated macrophages or TAM and tumor-associated fibroblasts or TAF (ex. cytokines, growth factors, ROS and other mediators) or in relation to hypoxic conditions. Very high levels of ROS can induce cancer cell injury and death whereas lower but sustained increase of ROS levels can result in new mutations and then potentially favor cancer progression. Upregulation of antioxidant genes, through NRF2 binding to target genes containing ARE sequences can favor survival of cancer cells. (B) In non-stressed conditions KEAP1 is bound to and maintain inactive both NRF2 and the two subunits of FACT complex, favoring their poly-ubiquitination and degradation. (C) In the presence of increased intracellular ROS levels, KEAP1 is detached from target proteins and this allows: i) NRF2 to bind to ARE sequences in antioxidant genes to up-regulate their transcription as well as to upregulate transcription of the subunits of FACT complex; ii) FACT complex to operate, through nucleosome assembly and disassembly, by inducing transcription elongation of NRF2 and of its target antioxidant genes. As a net result of this scenario liver cancer cells have an increased chance to survive to oxidative stress, overall favoring cancer progression. (D) Curaxin, by selectively inhibiting FACT complex, can suppress HCC growth and sensitizes liver cancer cells to the action of sorafenib, opening the way to a novel potential therapeutic strategy for HCC.